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Stereochemical Definition and Chirospecific Synthesis of the Peptide Deformylase Inhibitor Sch 382583

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Abstract: The recently reported natural product Sch 382583 (**1**), an inhibitor of peptide deformylase, has been synthesized in 16 steps from commercially available starting materials. The three chiral centers were set by a combination of chiral auxiliary and chiral pool approaches. The succinate **5** and piperazic acid **9** moieties were obtained by Evans oxazolidinone imide enolate alkylation and hydrazination/cyclization, respectively, and the aminohexanone side chain **13** was prepared via Grignard substitution of the Weinreb amide derived from L-valine. Spectroscopic data for the resulting synthetic material, compared with the data reported for the natural product, established that the previously unassigned valine ketone stereocenter (C-4) has the *S*-configuration.

Natural products, particularly those derived from microbial fermentation, have served as a rich source of drugs and agrochemicals, as well as lead structures in the search for bioactive molecules in general.[1] The naturally occurring antibacterial actinonin (Figure 1) has been identified as a potent inhibitor[2] of peptide deformylase (PDF), a hydrolase enzyme that catalyzes the cleavage of the formyl group at the N-terminal methionine residue of nascent polypeptides in bacterial protein translation.[3] As this process is not part of mammalian protein biosynthesis, PDF has been identified as a promising antimicrobial target.[4] Small-molecule inhibitors of this enzyme, representing over a dozen distinct chemical classes, have been discovered by means of (1) high-throughput screening of diverse compounds from private chemical databases, (2) screening of libraries of known metalloprotease inhibitors, and (3) design based on substrate or proposed transition-state structure.[5] Most of these inhibitors share with actinonin a hydroxamic acid moiety that binds to the active site iron cofactor of PDF, which is essential for enzymatic activity. However, the synthetic inhibitors, while potent against the isolated enzyme, have largely proven disappointing in terms of antibacterial activity when compared to actinonin and related analogues.[5f,6]

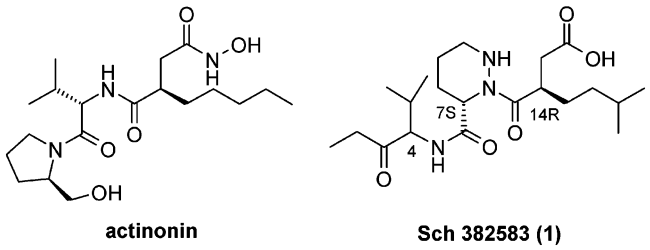

FIGURE 1. Natural product inhibitors of peptide deformylase.

Recognizing the value of natural products as potent, bioavailable PDF inhibitors, we became quite interested in a recent report from the Schering-Plough group describing the isolation and structure determination of Sch 382583 (**1**) from *Streptomyces*.[7] With a K_i of 60 nM, this piperazic acid-containing pseudopeptide is the most potent carboxylic acid inhibitor of PDF reported to date. While structurally similar to the matlystatins[8] as well as actinonin, Sch 382583 contains a unique succinamide side chain substituted by a 3-methylbutyl group. There are several features of this new natural product that make it an important target for synthesis. We viewed the conformational rigidity imparted by the piperazic acid ring as useful for evaluating the effect of ligand structure on enzyme activity. Also, unlike the hydroxamic acid-containing actinonin, Sch 382583 is a carboxylic acid. Because carboxylic acid inhibitors of a closely related class of enzymes, the matrix metalloproteases, are considerably less potent than the corresponding hydroxamic acids,[9] it seemed likely that the activity of Sch 382583 could be enhanced via modification of the metal-binding group.[10] Finally, on the basis of the X-ray structure of a

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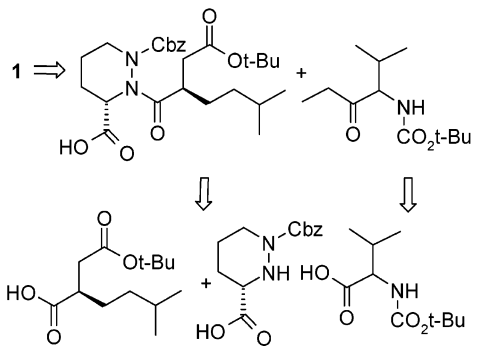
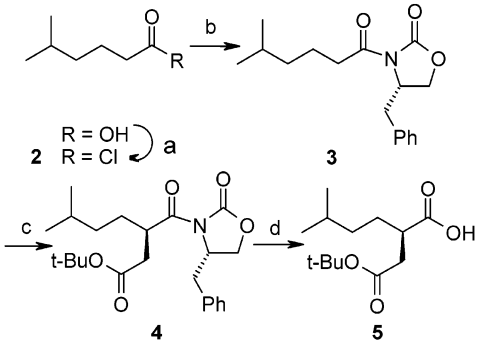


FIGURE 2. Retrosynthetic analysis of **1**.

SCHEME 1. Synthesis of the Chiral Succinate 5[a]



[a] Reagents: (a) $SOCl_2$, 80%; (b) (*S*)-(−)-4-benzyloxazolidinone, *n*-BuLi, THF, −78 °C, 49%; (c) $NaN(Si(CH_3)_3)_2$, *tert*-butyl bromoacetate, THF, −78 °C, 42%; (d) LiOH, H_2O_2, 99%.

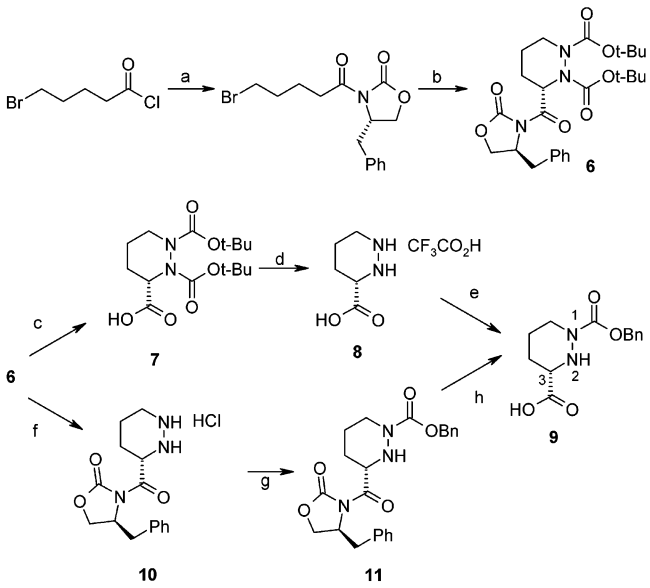
close analogue cocrystallized with PDF,[7] the stereochemistries of the asymmetric centers at C-7 and C-14 in Sch 382583 were inferred to be *S* and *R*, respectively. Because of the low resolution in that region of the inhibitor−enzyme complex, the stereochemistry at the final center, C-4, could not be established and remained unassigned until the present study.

The first total synthesis of **1** is reported herein, with assignment of absolute stereochemistry at all three chiral centers. Our synthetic approach involved a convergent synthesis utilizing the three major disconnects shown in Figure 2.

The required chiral succinic acid was prepared using the Evans oxazolidinone alkylation method as shown in Scheme 1.[11] Treatment of commercially available 5-methylhexanoic acid with thionyl chloride gave the acid chloride **2**,[12] which was reacted with the chiral auxiliary (*S*)-(−)-4-benzyloxazolidinone and *n*-BuLi in THF at −78 °C to yield the intermediate imide **3**. The imide was alkylated stereospecifically with *tert*-butyl bromoacetate by generation of the enolate anion with sodium hexamethyldisilazide to give **4**.

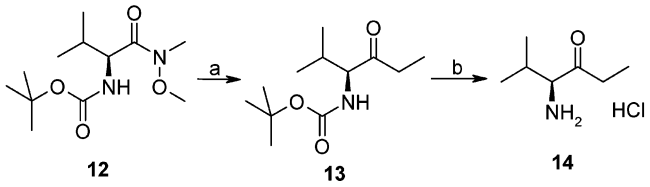
Cleavage of the chiral auxiliary of **4** to yield the monoprotected succinate **5** was performed under standard conditions. The optical purity of the product **5** was determined by conversion to diastereomeric amides with (*R*)- and (*S*)-phenylalanine methyl ester, respectively. HPLC co-injection of aliquots of the diastereomeric amide mixture formed from the (*S*)-amino ester with the amide mixture from the (*R*)-amino ester established the optical purity of the succinate **5** as >98% ee.

SCHEME 2. Synthesis of (3S)-*N*-Cbz-piperazic Acid (9)[a]



[a] Reagents: (a) *n*-BuLi, THF, (*S*)-(−)-4-benzyl-2-oxazolidinone, −78 °C, 85%; (b) LDA, di-*tert*-butyl azodicarboxylate, DMPU, −78 °C, 99%; (c) LiOH, 0 °C, 76%; (d) CF_3CO_2H, 66%; (a)−(d) see ref 14; (e) 1 N aq NaOH, $PhCH_3$, $ClCO_2Bn$, 40%, see ref 15; (f) HCl/dioxane, rt; (g) $(^iPr)_2NEt$, $ClCO_2Bn$, 0 °C, 69% (from **6**); (h) LiOH, 0 °C, 67%.

SCHEME 3. Synthesis of (*S*)-4-Amino-5-methyl-3-hexanone Hydrochloride (14)[a]



[a] Reagents: (a) EtMgBr, THF, 0 °C, 94%; (b) HCl, dioxane, rt, 83%.

Piperazic acid is a hydrazino amino acid found in many biologically active natural products.[13] To form the amide bond at N-2, we first needed to protect the more nucleophilic nitrogen (N-1) of piperazic acid. The carbobenzoxy (Cbz) protecting group was chosen because we anticipated its facile cleavage by hydrogenolysis as being an attractive option for the final step in the synthesis of Sch 382583. Preparation of the Cbz-protected piperazic acid (**9**) has been reported in both racemic and chiral forms. For our needs, we found that the chiral enolate hydrazination/cyclization route of Hale and co-workers, as shown in Scheme 2, provided sufficient quantities of the trifluoroacetate salt of the (3*S*)-piperazic acid (**8**).[14] In our hands, variable results were obtained when this salt was

(10) After the completion of our Sch 382583 synthesis, a report from the British Biotech group on modification of the metal-binding group of a synthetic PDF inhibitor, BB-3497, appeared: Smith, H. K.; Beckett, R. P.; Clements, J. M.; Doel, S.; East, S. P.; Launchbury, S. B.; Pratt, L. M.; Spavold, Z. M.; Thomas, W.; Todd, R. S.; Whittaker, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3595−3599.

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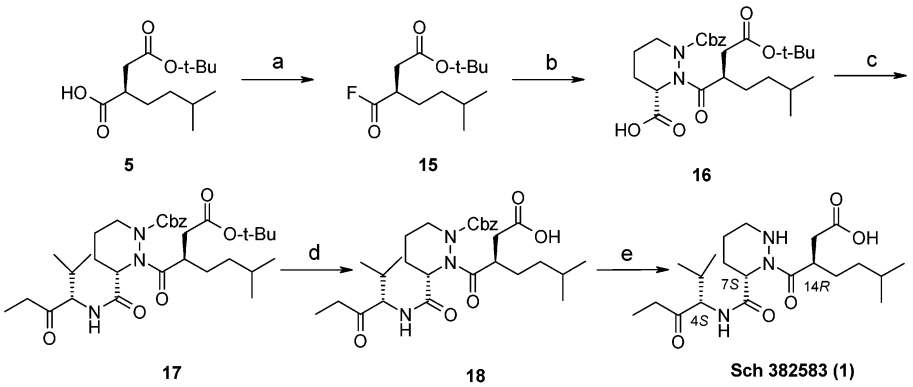
SCHEME 4. Assembly of 1[a]



[a] Reagents: (a) cyanuric fluoride, CH_2Cl_2, 5 °C, 91%; (b) $(^iPr)_2NEt$, **9**, rt to 45 °C, 34%; (c) $(EtO)_2P(O)CN$, **14**, 0 °C, 45%; (d) CF_3CO_2H, CH_2Cl_2, 80%; (e) 10% Pd−C, MeOH, rt, 94%.

converted to the protected form of the acid. Conditions set forth by Adams and co-workers[15] for conversion of the racemic piperazic acid were utilized in converting the chiral salt **8** to **9** in 40% yield. The low yield of this unoptimized reaction may be due to the water solubility of the product, possible side reaction at N-2, or other undetermined causes.

More conveniently, compound **9** can be obtained as shown in Scheme 2. The biscarbamate **6** was treated with HCl in dioxane to yield compound **10**. Treatment of compound **10** with Hunig's base and benzyl chloroformate resulted in compound **11** in 69% yield. Subsequent hydrolysis of the chiral oxazolidin-2-one of **11** afforded the desired **9** with an overall yield of 46% from compound **6**.

The third portion of the molecule was prepared as shown in Scheme 3 by reacting the Weinreb amide of Boc-L-valine (**12**) with ethylmagnesium bromide in THF to give the protected amino ketone **13**. Deprotection of **13** with anhydrous HCl gave the desired amino ketone **14** as the hydrochloride salt. The general knowledge that amino ketones are relatively unstable[16] prompted us to prepare **14** as needed from **13**. Synthesis of the amino ketone **14** has been reported previously via *N*-methyl-*O*-methyl-*N*-Cbz-valine.[17] However, we have found the commercially available Weinreb amide **12** to be equally serviceable.

The assembly of our three chiral building blocks is shown in Scheme 4. To avoid an additional protection/deprotection sequence, we decided to couple the free piperazic acid **9** to the succinate **5**.[18] Using the methodology developed by Carpino,[19] reaction of **5** with cyanuric fluoride gave the intermediate acid fluoride **15**, which was reacted with acid **9** to give succinamide **16**. This compound was treated with an excess of amino ketone **14** under basic conditions in the presence of diethyl phosphorylcyanidate[17] to give amide **17**.

Treatment of the *tert*-butyl ester **17** with trifluoroacetic acid yielded acid **18**, and catalytic hydrogenation to remove the benzyl carbamate of **18** provided **1** as a thick oil. The ^1H NMR and ^{13}C NMR spectra data of the synthetic material prepared in this study were identical to the data reported for the natural product by Chu and co-workers.[7]

In conclusion, this 16-step synthesis of **1**, a structurally unique inhibitor of the important antibacterial target peptide deformylase, establishes the absolute stereochemistry as *S* for the amino ketone side chain (C-4). Efficiency in the number of steps was achieved by direct coupling of the unprotected piperazic acid **9** to the succinate **5**. This convergent synthesis has proven amenable to the preparation of analogues that are useful for the establishment of structure−activity trends. Those results will be reported in due course.

Experimental Section

General Procedures. Melting points were obtained in open capillaries and are reported uncorrected. Reactions were carried out under an atmosphere of dry nitrogen. Organic extracts were dried with $MgSO_4$ before concentration at reduced pressure using a rotary evaporator. Specific rotations $[\alpha]_D$ were calculated from the observed rotations as a solution in methanol unless noted. All IR spectra were recorded as Nujol mulls unless noted. ^1H and ^{13}C NMR spectra were obtained on a 300 MHz instrument and are reported in parts per million in deuteriochloroform relative to tetramethylsilane (0.0 ppm) unless noted. An internal standard of $CFCl_3$ was used for ^{19}F spectra. Significant ^1H NMR data are tabulated in the order multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; br, broad), number of protons, and coupling constant(s) in hertz (Hz).

(*S*)-1-(1-Methylethyl)-2-oxobutyl)carbamic Acid 1,1-Dimethylethyl Ester (13). A 3.0 M solution of ethylmagnesium bromide in diethyl ether (25 mL, 75 mmol) was added at 0 °C to the Weinreb amide **12** (4.0 g, 15.4 mmol) in 35 mL of THF over 20 min, maintaining the internal temperature below 5 °C. The mixture was stirred for 4 h and then quenched by slow addition of 1 M HCl (10 mL) and diethyl ether (30 mL). The mixture was extracted into ether and washed with water and then brine, dried, filtered, and concentrated. The resulting oil was purified by column chromatography (10−30% EtOAc in hexanes to isolate the BOC-protected amino ketone **13** as a solid (3.0 g, 91%): mp 59−61 °C; $[\alpha]^{25}_D$ −13.9° (*c* 0.098); IR (Nujol) 1366, 1542, 1701, 1718, 3304 cm^{-1}; ^1H NMR δ 0.78 (d, 3H, J = 6.8), 1.01 (d, 3H,

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J = 6.5), 1.08 (t, 3H, J = 7.4), 1.44 (s, 9H), 2.19 (m, 1H), 2.51 (m, 2H), 4.28 (m, 1H), 5.15 (br d, 1H); ^{13}C NMR δ 7.7, 16.8, 19.9, 28.4 (3 C's), 30.5, 34.0, 63.9, 79.7, 156.1, 210.3. Anal. Calcd for $C_{12}H_{23}NO_3$: C, 62.61; H, 10.43; N, 6.09. Found: C, 62.77; H, 10.40; N, 6.12.

(S)-4-Amino-5-methyl-3-hexanone Hydrochloride (14). To a solution of BOC-protected amino ketone **13** (0.5 g, 2.2 mmol) in dioxanes (10 mL) was added a 3.6 M solution of HCl in dioxanes at rt in one portion. The mixture was stirred at rt for 6 h, then an additional 2 mL of 3.6 M HCl in dioxanes was added, and stirring was continued at rt for 48 h. Excess solvent was removed under reduced pressure, and the resulting solid was suspended in hexanes, filtered, and dried to give the desired amino ketone **14** (360 mg, 83%) as a white solid: mp 136−141 °C; $[\alpha]^{25}_D$ +37.8° (*c* 0.12); IR 1522, 1593, 1724 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.89 (d, 3H, J = 6.9), 0.99 (m, 6H), 2.32 (m, 1H), 2.64 (q, 2H, J = 7.2), 3.99 (d, 1H, J = 4.1), 8.5 (br s, 3H); ^{13}C NMR (DMSO-d_6) δ 5.7, 15.7, 17.3, 26.9, 31.9, 61.1, 205.6.

1,1-Dimethylethyl (3*R*)-3-(Fluorocarbonyl)-6-methylheptanoate (15). To a solution of succinate **5** (700 mg, 2.9 mmol) stirring in CH_2Cl_2 (10 mL) was added pyridine (0.24 mL, 2.9 mmol). This stirred mixture was cooled to 5 °C, and then cyanuric fluoride (1.25 mL, 15.0 mmol) was added in one portion. A white precipitate formed which slowly dissolved, and then a second precipitate formed. The suspension was then allowed to stir at rt for 18 h. The reaction mixture was filtered through a fine frit funnel, and the solid was rinsed with CH_2Cl_2. The filtrate was partitioned between CH_2Cl_2 and chilled water. The organic layer was separated and washed with brine, dried, filtered, and concentrated to give the acid fluoride **15** (650 mg, 91%) as an oil: IR (neat) 1730, 1838 cm^{-1}; ^{1}H NMR δ 0.85 (d, 6H, J = 6.6), 1.18 (m, 2H), 1.39 (s, 9H), 1.55 (m, 3H), 2.50 (m, 1H), 2.59 (m, 1H), 2.95 (m, 1H); ^{19}F NMR (282.2 MHz) δ 38.39 (s).

1-(2-Phenylmethyl) Hydrogen (3*S*)-2-(2*R*)-2-[2-(1,1-dimethylethoxy)-2-oxoethyl]-5-methyl-1-oxohexyl] Tetrahydro-1,3(2*H*)-pyridazinedicarboxylate (16). To 480 mg (1.8 mmol) of (3*S*)-*N*-Cbz-piperazic acid (**9**) in CH_2Cl_2 (20 mL) was added *N*,*N*-diisopropylethylamine (410 μL, 2.5 mmol) in one portion followed by a solution of acid fluoride **15** (450 mg, 1.8 mmol) in CH_2Cl_2 (10 mL). The resulting solution was stirred at rt for 56 h and then heated to 45 °C for 1 h. The mixture was cooled to rt and then partitioned between CH_2Cl_2 and water. The organic layer was separated, washed with water and brine, dried, filtered, and concentrated to give a crude oil (760 mg). This oil was purified by silica gel chromatography (1−5% AcOH in 1:1 hexanes/EtOAc) to isolate amide **16** (300 mg, 34%) as an oil: $[\alpha]^{26}_D$ −20.6° (*c* 0.081); IR (neat) 1720, 1722, 3100 cm^{-1}; ^{1}H NMR (signals broadened due to hindered rotation) δ 0.80 (m, 6H), 1.15 (m, 2H), 1.27 (m, 2H), 1.39 (m, 11H), 1.70 (m, 2H), 1.95 (m, 3H), 2.39 (dd, 1H, J = 3.6, 16.9), 2.70 (m, 1H), 3.10 (m, 1H), 3.80 (m, 1H), 4.10 (m, 1H), 5.21 (d, 2H, J = 13), 7.35 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.1, 22.3 (2C), 22.8, 23.3, 28.0, 28.2 (3C), 29.3, 36.1, 37.0, 37.3, 46.0, 56.1, 69.7, 80.9, 128.6 (2C), 128.8 (2C), 135.0, 159.0, 171.7, 172.2, 179.7. Anal. Calcd for $C_{26}H_{38}N_2O_7$: C, 63.65; H, 7.81; N, 5.71. Found: C, 63.40; H, 7.83; N, 5.50.

1,1-Dimethylethyl (*βR*,6*S*)-tetrahydro-*β*-(3-methylbutyl)-6-[[[(1*S*)-1-(methylethyl)-2-oxobutyl]amino]carbonyl]-*γ*-oxo-2-[(phenylmethoxy)carbonyl]-1(2*H*)-pyridazinebutanoate (17). To 200 mg (0.40 mmol) of piperazic acid **16** stirring in 8 mL of a 3:1 mixture of THF/DMF was added 170 μL (1.2 mmol) of triethylamine in one portion. This mixture was cooled to 0 °C, and then the amino ketone hydrochloride **14** (200 mg, 1.2 mmol) was added in one portion, followed by diethyl phosphorylcyanidate (190 μL, 1.2 mmol) in one portion. The mixture was allowed to warm to rt, stirred for 16 h, and then partitioned between EtOAc and H_2O. The organic layer was separated, washed with brine, dried, filtered, and concentrated to give an oil. This oil was purified by silica gel chromatography (30−50% EtOAc/hexanes) to isolate 110 mg (45%) of **17** as a clear oil: $[\alpha]^{25}_D$ −25.3° (*c* 0.14); ^{1}H NMR (400 MHz, $CDCl_3$, signals broadened due to hindered rotation, rotamers) δ 0.80 (m, 12H), 0.85 (m, 2H), 1.02 (t, 3H, J = 7.2), 1.15 (m, 1H), 1.20 (m, 1H), 1.40 (s, 9H), 1.70 (m, 6H), 2.02 (m, 1H), 2.13 (m, 1H), 2.50 (m, 2H), 2.85 (m, 1H), 2.95 (m, 1H), 3.80 (m, 1H), 4.40 (m, 1H), 5.10 (m, 1H), 5.20 (m, 2H), 7.40 (m, 5H), 8.22 (d, 1H, J = 8.4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.6, 17.8, 19.6, 19.9, 20.1, 22.5, 22.9, 28.2, 28.3 (3C), 29.6, 30.0, 34.0, 36.3, 37.3, 37.5, 46.0, 57.9, 63.5, 68.9, 69.1, 80.8, 128.2 (2C) 128.5, 128.8 (2C), 157.5, 170.9, 172.2, 179.8, 210.0; HRMS *m*/*z* calcd for $C_{33}H_{52}N_3O_7$ $(M + H)^+$ 602.3805, found 602.3795.

(*βR*,6*S*)-Tetrahydro-*β*-(3-methylbutyl)-6-[[[(1*S*)-1-(methylethyl)-2-oxobutyl]amino]carbonyl]- *γ*-oxo-2-[(phenylmethoxy)carbonyl]-1(2*H*)-pyridazinebutanoic Acid (18). To *tert*-butyl ester **17** (110 mg, 1.8 mmol) in CH_2Cl_2 (15 mL) was added 0.25 mL (3.3 mmol) of trifluoroacetic acid. This mixture was heated at reflux for 2 h and cooled, then more trifluoroacetic acid (0.25 mL) was added, and reflux was continued for 4 h. This addition and heating sequence was repeated two more times, then the reaction was cooled, and the volatiles were removed under reduced pressure to give an oil. This oil was partitioned between EtOAc and saturated aqueous $NaHCO_3$. The organic layer was separated, washed with brine, dried, filtered, and concentrated to give 80 mg (80%) of **18** as an oil which solidified upon standing: mp 116−120 °C; $[\alpha]^{25}_D$ −23.5° (*c* 0.35); IR (neat) 1404, 1706, 2960, 3400 cm^{-1}; ^{1}H NMR δ 0.80 (m, 12H), 1.02 (m, 3H), 1.15 (m, 2H), 1.20 (m, 2H), 1.45 (m, 1H), 1.70 (m, 3H), 2.13 (m, 1H), 2.50 (m, 3H), 2.85−2.95 (m, 2H), 3.65 (m, 1H), 4.15 (m 1H), 4.40 (m, 1H), 4.60 (m 1H), 4.96 (m, 1H), 5.20 (m, 2H), 7.36 (m, 5H), 8.15 (d, 1H); ^{13}C NMR δ 7.5, 17.6, 19.6, 19.8, 22.4, 22.7, 23.4, 28.1, 29.4, 30.0, 33.9, 35.7, 36.1, 37.5, 46.0, 58.5, 63.6, 69.1, 128.2 (2C), 128.4, 128.8 (2C), 135.5, 157.8, 171.4, 176.6, 179.9, 209.9; MS AP(+) *m*/*z* 546.12 $(M + H)^+$, AP(−) *m*/*z* 544.04 $(M - H)^-$; HRMS *m*/*z* calcd for $C_{29}H_{44}N_3O_7$ $(M + H)^+$ 546.3179, found 546.3168.

(*βR*,6*S*)-Tetrahydro-*β*-(3-methylbutyl)-6-[[[(1*S*)-1-(methylethyl)-2-oxobutyl]amino]carbonyl]-*γ*-oxo-1(2*H*)-pyridazinebutanoic Acid (1). Acid **18** (70 mg, 1.3 mmol) in methanol (10 mL) was combined with 70 mg of 10% palladium on carbon at rt under a blanket of nitrogen. A balloon of hydrogen was placed over the reaction mixture, and it was stirred for 6 h. The reaction was purged with nitrogen and filtered through a pad of Celite, rinsing with methanol. The solvent was removed under reduced pressure to give an oil that was purified by silica gel chromatography (1−5% AcOH in 1:1 hexanes/EtOAc) to give the desired product **1** as an oil (50 mg, 94%): $[\alpha]^{25}_D$ −22° (*c* 0.051); ^{1}H NMR δ 0.71 (d, 3H, J = 6.6), 0.77 (d, 6H, J = 6.6), 0.88 (d, 2H, J = 6.6), 1.01 (t, 3H, J = 7.5), 1.11 (m, 2H), 1.43 (m, 6H), 2.0 (m, 1H), 2.15 (m, 2H), 2.41 (m, 3H), 2.75 (m, 2H), 2.94 (d, 1H, J = 11.9), 3.76 (m, 1H), 4.50 (m, 2H), 5.19 (m, 1H), 6.86 (d, 1H, J = 8.4); ^{13}C NMR δ 7.7, 17.2, 20.1, 21.1, 22.4, 22.8, 25.9, 28.1, 30.1, 30.3, 34.2, 35.9, 36.3, 36.5, 47.3, 50.8, 62.7, 172.1, 177.0, 178.1, 209.8; HRMS *m*/*z* calcd for $C_{21}H_{38}N_3O_5$ $(M + H)^+$ 412.2726, found 412.2812.

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Supporting Information Available: Experimental procedures for compounds **2**−**5** and **9**−**11**, optical purity determination of compounds **5** and **9**, and a table of ^{1}H and ^{13}C NMR spectroscopic data for comparison of the data of synthetic **1** to the data reported for the natural product (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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